

Enrichment of outgrowth endothelial cells from human peripheral blood by protocol modification

M. Kolbe, S. Fuchs, D. Katerla, B. Pavic, C.J. Kirkpatrick

Institute of Pathology, Johannes Gutenberg University Mainz, Germany

INTRODUCTION: Results of several studies showed that outgrowth endothelial cells (OEC), defined as a subpopulation of endothelial precursor cells (EPC), isolated from human peripheral blood, promote neovascularisation [1, 2]. For therapeutical approaches in vitro expansion of OEC is necessary [3, 4]. A reduction of expansion time as well as a higher number of OEC gained per individual donor might promote the clinical application of OEC in future. In this study two different protocols for the isolation of OEC were compared with the aim to develop a more effective procedure for OEC isolation.

METHODS: Human peripheral blood mononuclear cells (MNC) were isolated by ficoll density centrifugation from buffy coats as previously published [3, 4]. This standard protocol was modified by adding a passaging step 7 days after isolation of MNC. Cells from standard and modified protocol were cultivated in EGM-2 Bullet Kit supplemented with 5% fetal calf serum and 1% Pen/Strep. 28 days after isolation the number of OEC colonies gained by both protocols was determined. At the same time endothelial marker expression was investigated by quantitative real-time PCR and flow cytometry for the CD31, vWF, CD146 and KDR and the endothelial precursor marker CD34 and CD133. Cell populations obtained by individual protocols were morphologically investigated by immuno-fluorescence staining for the endothelial markers CD31 and vWF.

RESULTS: MNC of 36 donors were isolated according to standard or modified protocol. 28 days after the isolation cell cultures were still heterogeneous. Using the modified protocol one group of donors (group A) showed only a minimal increase in OEC colonies, whereas the other group (group B) showed a significant increase in OEC colony formation (tab.1). Independent of the isolation procedure cultures from group B formed significantly more OEC colonies than cultures of group A. Quantitative real time PCR and flow cytometry and immunofluorescence staining (fig.1) indicate an

enrichment of OEC by the modified protocol. At the same time cells of group B showed an upregulated expression of the endothelial precursor marker CD34 and CD133 compared with donors of group A (fig.2).

Table 1. Numbers of observed OEC 28 days after isolation (*significant $p < 0,03$)

	Number of OEC colonies per $2,4 \times 10^8$ seeded MNC	
	standard protocol	modified protocol
group A (n= 25)	2,8 ± 2,1	3,4 ± 2,7
group B (n=11)	5,1* ± 2,7	28,8* ± 12,7

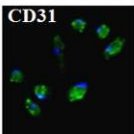
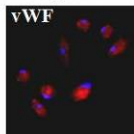
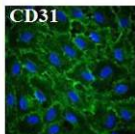
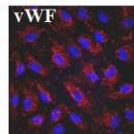
Cells from standard protocol		Cells from modified protocol	
			

Fig. 1: Culture morphology 28 days after isolation according to standard and modified protocol (n=4)

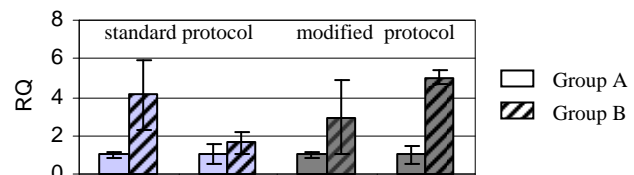


Fig. 2: Expression of the precursor marker CD34 and CD133 in cultures 28 days after isolation (n=4)

DISCUSSION & CONCLUSIONS: The modified protocol, including a passaging step showed an enrichment of OEC in heterogeneous culture from MNC 28 days after isolation for donors of group B. On the other hand increased levels of EPC markers in cells of group B suggest that the modified protocol might favour an enrichment of OEC with stem cell characteristics.

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